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## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference | FOR FURTHER ACTION | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| E SD/RS/VV20/2 | | |
| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
| PCT/NL99/00504 | 06/08/1999 | 14/08/1998 |
| International Patent Classification (IPC) or national classification and IPC | | |
| G01N33/543 | | |
| Applicant | | |
| HOLLAND BIOMATERIALS GROUP B.V. et al. | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 1 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 14/03/2000 | 14.11.2000 |
| Name and mailing address of the international preliminary examining authority:<br>European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Authorized officer<br>Vanmontfort, D<br>Telephone No. +49 89 2399 8457 |

Form PCT/IPEA/409 (cover sheet) (January 1994)

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NL99/00504

## I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
**Description, pages:**

| | | | | |
|---|---|---|---|---|
| 1-16 | as originally filed | | | |

**Claims, No.:**

| | | | | |
|---|---|---|---|---|
| 2-8,10-24 | as originally filed | | | |
| 1,9 | as received on | 20/10/2000 | with letter of | 20/10/2000 |

**Drawings, sheets:**

| | | | | |
|---|---|---|---|---|
| 1/1 | as originally filed | | | |

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. Th amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   *(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 1-24 |
| | No: | Claims | |
| Inventive step (IS) | Yes: | Claims | 1-22, 24 |
| | No: | Claims | 23 |
| Industrial applicability (IA) | Yes: | Claims | 1-24 |
| | No: | Claims | |

2. Citations and explanations
   **see separate sheet**

**VII. Certain defects in the international application**

The following defects in the form or contents of the international application have been noted:
**s e separate sheet**

**VIII. Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
**see separate sheet**

## 1. Section V

Reference is made to the following documents:

D1 EP 0 104 608 A (BECTON DICKINSON CO) 4 April 1984
D2 WO 97 38801 A (BOARD OF REGENTS; UNIVERSITY OF TEXAS) 23 October 1997
D3 US 5 723 219 A (JOHANSON ROBERT G ET AL) 3 March 1998
D4 US 5 627 079 A (GARDELLA JR JOSEPH A ET AL) 6 May 1997

1.1 The subject-matter of claims 1-8 and 20 is novel and inventive (Article 33(2) and 33(3) PCT).
D4 (abstract; claims 1, 8 and 15), which is considered to represent the most relevant state of the art, discloses the fluorination of surfaces by gas phase surface fluorination or plasma deposition. The document further discloses the use of a metallic layer such as gold, nickel, ... .
The subject-matter of claim 1 differs from D4 in that sulphur is comprised in the plasma deposited layer. Furthermore, the combination of **a free electron metal film consisting essentially of gold** and a plasma layer deposited on the gold film, which **plasma layer comprises sulphur** is not disclosed in any of the available prior art documents. The problem to be solved can therefore be formulated as the provision of an improved device for investigating the reactions between interactive chemical species. Although the use of a sulphur compound containing plasma layer is disclosed in D1 (claim 1), D2 (claim 9) and D3 (claim 3), there is no indication in any of the available prior art documents that the combination of a gold film and a plasma deposited layer thereon comprising sulphur leads to a more uniform, homogeneous and stable functional surface layer. Therefore, it would not be obvious for a person skilled in the art to make this combination because none of the available prior art documents teaches or hints that the above-mentioned combination leads to an improved stability of the surface. Hence, claim 1 is considered to involve an inventive step (Article 33(3) PCT). The same applies to claims 2-8 and 20.

1.2 The subject-matter of claims 9-19, 21 and 22 is novel and inventive (Article 33(2) and 33(3) PCT). The process to provide the device of claim 1 (claims 9-19),

process for investigating the interaction of chemical and/or biological species on a device of claim 1 (claim 21) and use of a device for investigating the reaction between chemically interactive species (claim 22) are novel and inventive given that the subject-matter of claim 1 is novel and inventive.

1.3 The subject-matter of claim 23 is not inventive (Article 33(3) PCT).
D2 (abstract; claims 1, 4 and 6) describes the process to deposit a reactive functional group on the surface of a solid substrate. The substrate to be modified includes polymer, metal, ... (claim 11) and a reactive functional group such as a sulphur compound (claim 9) can be used. The interaction at the surface was afterwards analysed by ESCA analysis (page 23 line 3).
The subject-matter of claim 23 differs from D2, which is considered to represent the most relevant prior art, in the following feature:
**the interaction between the functional groups is investigated by surface plasmon resonance spectroscopy (SPR)**
The problem to be solved by the present invention may therefore be regarded as the provision of an alternative method to analyze interactions between different functional groups. A person skilled in spectroscopy would know which techniques are available to investigate surface interactions. It would be obvious for a skilled person to use the SPR technique instead of the ESCA technique to solve the problem posed. Therefore, the subject-matter of claim 23 does not involve an inventive step.

1.4 The subject-matter of claim 24 is novel and inventive (Article 33(2) and 33(3) PCT) for the same reasons as explained in point 1.1 above.

## 2. Section VII

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D2 and D4 are not mentioned in the description, nor are these documents identified therein.

## 3. Section VIII

3.1 The claims are not supported by the description as required by Article 6 PCT.

References to claims in the description are not sufficient as support for said claims.

3.2 Although claims 1-5 and 7 have been drafted as separate claims, they appear to relate effectively to the same subject-matter. Therefore, claims 2-5 and 7 are superfluous (Article 6 PCT).

3.3 The unit "bar" employed in claim 13 and throughout the description and the unit "sccm/min" employed in the examples are not recognized in international practice, contrary to the requirements of Rule 10.1(d) PCT.

3.4 Independent claim 18 includes all the features of claim 17. Hence, claim 18 should be reformulated as a claim dependent of claim 17 (Rule 6.4 PCT and the PCT Guidelines C-III, 3.5). The only different feature in claim 18 is that said free electron metal substrate is preferably gold and that said functional group species is preferably selected from a sulphur compound.

3.5 The term "surface plasmon resins spectroscopy" used in claim 23 is vague and unclear and leaves the reader in doubt as to the meaning of the technical features to which it refers, thereby rendering the definition of the subject-matter of said claim/s unclear (Article 6 PCT). This term should be replaced by "surface plasmon resonance spectroscopy".

Int.pat.appln. PCT/NL99/00504
Enc. to letter dated 20 October 2000

(68)

**NEW CLAIMS**

1. Device for investigating reactions between interactive chemical and/or biological species, said device comprising:

- a substrate and
- a plasma layer deposited on the substrate,

characterized in that the substrate in turn comprises a film of free electron metal consisting essentially of gold, and wherein the plasma layer deposited on the film of free electron metal comprises sulphur.

9. Process for providing a device for investigating reactions between interactive chemical an biological species, said process comprising the steps of providing a preselected substrate, which substrate in turn comprises a film of free electron metal consisting essentially of gold and arranging a layer on the gold film by plasma deposition, which layer comprises sulphur.

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From the INTERNATIONAL BUREAU

## PCT

### NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

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| **Applicant**<br>TERLINGEN, Johannes, Gijsbertus, Antonius et al | |

1. The designated Office is hereby notified of its election made:

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**Published**
*Without international search report and to be republished upon receipt of that report.*

(54) Title: DEVICE FOR INVESTIGATING CHEMICAL INTERACTIONS AND PROCESS UTILIZING SUCH DEVICE

(57) Abstract

The invention relates to a device for investigating reactions between interactive species, said device comprising: one or more plasma deposited layers, which layers comprise one or more first pre–selected functional group species, which functional group species are interactible with a pre–selectable second species.

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# DEVICE FOR INVESTIGATING CHEMICAL INTERACTIONS AND PROCESS UTILIZING SUCH DEVICE

The present invention relates to a device for investigating reactions between interactive chemical and/or biological species, to a process for providing such a device, and to a process for investigating chemical and/or biological interactions, for example biomolecular interactions, utilizing such a device.

Under chemical and/or biological interactions is also understood chemical and/or biological reactions.

Interactions of specific compounds with solid surfaces play a crucial role in chemical and biological phenomena and areas including analysis techniques such as RIA's, ELISA's.

For investigating and sensing surface interactions a 'sensitive' surface is required.

To study real time surface interactions several techniques are available such as ellipsometry, reflectometry and surface plasmon resonance spectroscopy (SPR). These techniques have in common that they use the reflectance of light, generated by a laser, to analyze the growth or desintegration of a layer of for instance biological molecules at a surface.

For these techniques, a reflecting surface is necessary. In the case of SPR, a surface comprising a free electron metal for example gold is most frequently used.

In order to utilize this technique for investigating other interactions, besides the interaction of (bio)molecules with free electron metal surfaces, the free electron surfaces have been modified, for instance, by the adsorption of bio-molecules such as proteins and the coating thereof with polymeric layers in a solvent cast or spin coat procedures.

Methods have also been developed to provide gold surfaces with specific chemical groups for the

immobilization of proteins, which surfaces are subsequently utilized for studying the interactions with other (biological) substances such as antibody-antigen interactions.

Methods for generating SPR sensor surfaces include arranging an organic surface onto a gold layer by means of a wet chemistry procedure such as solvent casting or spin coating before carrying out a plasma etching procedure.

A further method includes adsorption of a chemical functional surfactant, by means of a wet chemistry procedure, on the surface to be modified and the subsequent immobilization of the surfactant by a plasma such as an argon plasma, so called plasma immobilization.

Disadvantages of these known techniques include the lack of stability of the functional surface layers.

An object of the present invention is to provide an improved device for investigating the reactions between interactive chemical species.

According to a first aspect of the present invention there is provided a device according to any of the claims 1 to 8.

The device according to the present invention provides a good attachment of the plasma deposited layer, a good stability thereof and a device exhibiting good sensitivity, whereby the substrate is provided with a functional layer, the functionality of which can be provided by groups such as amine, carboxylic acid, hydroxyl, acid chloride, isocyanate, aldehyde, anhydride, epoxide, and thiol groups for example.

According to a second aspect of the present invention, there is provided a process according to any of the claims 9 to 19 for providing the device according to the present invention.

Since a functional group layer is plasma deposited, control over the deposition thereof can be accurately carried out, whereby very thin layers can be

deposited thus providing very sensitive devices, without the need for firstly arranging an organic layer by wet chemical methods on the substrate before any further investigation can be carried out.

The process according to the present invention provides a good controllability.

In contrast to processes for providing sensor devices, wherein layers are arranged on a substract by wet chemical processes which are often time consuming, difficult to carry out, and often result in undesirably thick layers exhibiting a subsequent lack of sensitivity if a great deal of care in not applied, the process according to the present invention is extremely flexible to work and easy to effect and offers a good cost efficiency.

Plasma deposition procedures involve the deposition of organic species from the plasma phase on a substrate. For instance by applying a (volatile) monomer as the gas phase an organic layer the structure of which resembles the corresponding polymer can be deposited. By applying a (volatile) monomer that possesses a chemical functionality a chemical functional polymeric layer can be obtained.

The plasma may be deposited from a monomer preferably being selected from the group consisting essentially of:

- unsaturated monomers; acrylic acid, allyl amine, allyl isocyanate, allyl mercaptan, methacrylic acid, allyl alcohol, allyl acetate, allyl acetic acid, allyl glycidyl ether, 3 allyloxy, 1-2 propanediol, vinyl acetate, acrylic acid halides,
- saturated monomers; alcohols such as methanol, ethanol propanol, acids such as propionic acid, acetic acid and the like, formaldehyde, propionic aldehyde, glutardialdehyde, aminoethane, aminoethanol, ethylene oxide, acetone methane, ethane, propane and the like, whereby the substrate is provided with the corresponding functionality.

Apart from the plasma deposition of saturated and unsaturated monomers, a functionality can be created in situ, i.e. in the plasma layer, by means of rearrangements of (cyclic) monomers or reaction between a mixture of plasma gases for example, whereafter this in-situ created functionality can be deposited.

Surfaces with a high surface energy, such as metal surfaces in general, may give rise to a rapid surface hydrophobisation due to contamination of the surface by species from its environment. This surface contamination may be disastrous for further surface modification for instance with respect to the stability of the final surface. Therefore this surface contamination should be prevented as much as possible by storing the surfaces in an inert atmosphere and reduction of the time between surface preparation and modification or the surface needs to be cleaned before modification. Plasma etching offers an excellent method for this cleaning. Plasma cleaning is fast and is a clean process in itself since it does not involve the use of organic solvent or substantial amounts of reagents that may have adverse effects on the environment. For the present invention it is advantageous to include an in situ plasma cleaning step of the substrate before the actual modification by plasma deposition.

The plasma deposited layer preferably comprises one or more sulphur compounds, for example thiols, sulfides and/or disulfides, i.e. in the form of mercaptoacetic acid, 2-mercaptopropionic acid, 3-mercaptopropionic acid, 1-mercaptopropenol, 2-mercaptoethanol and the like, preferably diallylsulfide, since, especially when gold is chosen as the substrate, an improved stability is provided.

According to a further aspect of the present invention there is provided a process for investigating the interaction of chemical and/or biological species, for example real time surface interactions, according to claims 14 or 15.

The invention will now be further clarified by way of the following examples, with reference to figure 1 which graphically shows the immobilization of albumins onto a COOH disk as carried out in example 12.

**Example 1**

**Preparation of carboxylic acid functional gold surfaces.**

Gold coated glass discs (60) were placed in the central position of the plasma reactor which consisted of a glass tubes (l = 150 cm, o= 10 cm) with three electrodes positioned at the outside of the glass tube with the powered electrode in the center and two grounded electrodes positioned at 30 cm distance on both sides of the powered electrode. The electrodes were connected to an RF-generator (13.56 MHz, ENI ACG-3, ENI Power Systems) through a matching network (ENI Matchwork 5) and a matching network control unit (ENI TH-1000, ENI). The generator was controlled by a timer (Apple Ile computer with a time control program).

The reactor was evacuated to a pressure less than 0.001 mbar by a rotary pump (DUO 004 B, Pfeifer) which was equipped with a filter (ONF 025, Pfeifer) to prevent oil back streaming. The pressure was measured by a pressure gauge (Baratron 628A01MDE, MKS Instruments) and read from a display module (PR4000, MKS Instruments). An air flow of 5 sccm/min resulting in a pressure of about 0.12 mbar, was established for 5 minutes after which the discs were treated with a dynamic air plasma (85 W) for 1 minute at the same flow conditions. Air flow was controlled by a mass flow controller (type 1259 + PR3000 control unit, MKS Instruments). After the plasma treatment the air flow was continued for 2 minutes and then stopped and an acrylic acid flow was established through the reactor via a direct monomer inlet resulting in a pressure of about 0.03 mbar. To prevent the acrylic acid to reach the pump after leaving the reactor, the acrylic acid flow was bypassed through a cold trap that was cooled with liquid nitrogen. The temperature of the

acrylic acid in the storage container was room temperature. After two minutes the surfaces were treated with 5 pulses of an acrylic acid plasma at a discharge power of 75 (W), the pulses being separated from each other by 30 seconds of acrylic acid flow through the reactor. After the final pulse the surface were exposed to 2 additional minutes of acrylic acid flow whereupon the acrylic acid flow was stopped and the reactor was brought to atmospheric pressure with air.

**Example 2**

**Preparation of amine functional surfaces**

Gold coated glass discs (60) were placed in the plasma reactor as described in example 1. The reactor was evacuated to a pressure of less than 0.05 mbar and an air flow of 5 sccm/min was established for 5 minutes whereupon the discs were treated with a dynamic air plasma (85 W) for 1 minute at the same flow conditions. Then air flow was stopped and an allyl amine flow (0.07 mbar) was established through the reactor the temperature of the monomer storage container was 36°C. After two minutes the surfaces were treated with 10 pulses of an allyl amine plasma at a discharge power of 75 W separated from each other by 10 seconds of allyl amine flow through the reactor. After the final pulse the surfaces were exposed to 2 additional minutes of allyl amine flow after which the allyl amine flow was stopped and the reactor was brought to atmospheric pressure with air.

**Example 3**

Gold coated substrates (6) were placed in the plasma reactor (see example 2) between the cold electrode on the gas inlet side of the reactor and the hot electrode. The reactor was evacuated to a pressure less than 0.005 mbar and an air flow of 5 sccm was established through the reactor. After 2 minutes of air flow the substrates were treated with a dynamic air plasma (5 sccm, 85 W) for 1 minute and subsequently exposed to an

air flow of 5 sccm for 10 minutes again. Then the air flow was stopped and after evacuation of the reactor, an allylamine flow at a pressure of 0.095 mbar was established through the reactor. After two minutes allylamine flow the substrates were exposed to ten pulses of 1 second of an allylamine plasma at a discharge power of 85 W, the pulses being separated by ten seconds allylamine flow. After the final allylamine plasma pulse the allylamine flow was continued for 2 minutes after which the flow was discontinued, the reactor was evacuated and subsequently brought to atmospheric pressure with air. Following, the surfaces were analyzed for carbon, oxygen, nitrogen and gold by X-ray photo-electron spectroscopy, of which the results are shown in the table below. Also surfaces that were rinsed with water for 1 hr and subsequently dried were analyzed by XPS.

Table 1

| element | surface composition (at%0) | |
|---|---|---|
| | before rinsing | after rinsing |
| C | 65.4 | 62.4 |
| O | 10.3 | 10.5 |
| N | 17.5 | 13.6 |
| Au | 6.8 | 13.4 |

**Example 4**

Gold coated substrates (6) were placed in the plasma reactor (see example 2) between the cold electrode on the gas inlet side of the reactor and the hot electrode. The reactor was evacuated to a pressure of less than 0.005 mbar and an argon flow of 5 sccm was established through the reactor. After 2 minutes of argon

flow the substrates were treated with a dynamic argon plasma (5 sccm, 85 W) for 1 minute and subsequently exposed to an argon flow of 5 sccm for 10 minutes again. Then the argon flow was stopped and after evacuation of the reactor, an allylamine flow at a pressure of 0.095 mbar was established through the reactor. After two minutes allylamine flow the substrates were exposed to ten pulses of 1 second of an allylamine plasma at a discharge power of 85 W, the pulses being separated by ten seconds allylamine flow. After the final allylamine plasma pulse the allylamine flow was continued for 2 minutes after which the flow was discontinued, the reactor was evacuated and subsequently brought to atmospheric pressure with air.

**Example 5**

Gold coated substrates (6) were placed in the plasma reactor (see example 2) between the cold electrode on the gas inlet side of the reactor and the hot electrode. The reactor was evacuated to a pressure of less than 0.005 mbar and an air flow of 5 sccm was established through the reactor. After 2 minutes of air flow the substrates were treated with a dynamic air plasma (5 sccm, 85 W) for 1 minute and subsequently exposed to an air flow of 5 sccm for 10 minutes again. Then the air flow was stopped and after evacuation of the reactor, an allylamine flow at a pressure of 0.095 mbar was established through the reactor. After two minutes allylamine flow the substrates were exposed to five pulses of 1 second of an allylamine plasma at a discharge power of 170 W, the pulses being separated by ten seconds allylamine flow, followed by five pulses of an allylamine plasma at a discharge power of 85 W, again the pulses being separated by ten seconds allylamine flow. After the final allylamine plasma pulse the allylamine flow was continued for 2 minutes after which the flow was discontinued, the reactor was evacuated and subsequently brought to atmospheric pressure with air. Following, the

surfaces were analyzed for carbon, oxygen, nitrogen and gold by X-ray photo-electron spectroscopy, of which the results are shown in the table below.

Table 2

| element | surface composition (atomic %) |
|---|---|
| C | 62.8 |
| O | 9.8 |
| N | 20.8 |
| Au | 6.6 |

**Example 6**

Gold coated substrates (6) were placed in the plasma reactor (see example 2) between the cold electrode on the gas inlet side of the reactor and the hot electrode. The reactor was evacuated to a pressure of less than 0.005 mbar and an air flow of 5 sccm was established through the reactor. After 2 minutes of air flow the substrates were treated with a dynamic air plasma (5 sccm, 85 W) for 1 minute and subsequently exposed to an air flow of 5 sccm for 10 minutes again. Then the air flow was stopped and after evacuation of the reactor, a mixed flow of allylamine and octadiene (66 v% allylamine) at a pressure of 0.055 mbar was established through the reactor. After two minutes allylamine/octadiene flow the substrates were exposed to ten pulses of 1 second of an allylamine/octadiene plasma at a discharge power of 85 W, the pulses being separated by ten seconds allylamine/octadiene flow. After the final plasma pulse the allylamine/octadiene flow was continued for 2 minutes after which the flow was discontinued, the reactor was evacuated and subsequently brought to atmospheric pressure with air. Following, the surfaces were analyzed for carbon, oxygen, nitrogen and gold by X-

ray photo-electron spectroscopy, of which the results are shown in the table below.

Table 3

| element | surface composition (atomic %) |
|---|---|
| C | 73.1 |
| O | 7.9 |
| N | 11.7 |
| Au | 7.3 |

**Example 7**

Gold coated substrates (6) were placed in the plasma reactor (see example 2) between the cold electrode on the gas inlet side of the reactor and the hot electrode. The reactor was evacuated to a pressure of less than 0.005 mbar and an air flow of 5 sccm was established through the reactor. After 2 minutes of air flow the substrates were treated with a dynamic air plasma (5 sccm, 85 W) for 1 minute and subsequently exposed to an air flow of 5 sccm for 10 minutes again. Then the air flow was stopped and after evacuation of the reactor, a mixed flow of allylamine and diallylsulfide (66 v% allylamine) at a pressure of 0.065 mbar was established through the reactor. After two minutes allylamine/diallylsulfide flow the substrates were exposed to ten pulses of 1 second of an allylamine/diallylsulfide plasma at a discharge power of 85 W, the pulses being separated by ten seconds allylamine/diallylsulfide flow. After the final plasma pulse the allylamine/diallylsulfide flow was continued for 2 minutes after which the flow was discontinued, the reactor was evacuated and subsequently brought to atmospheric pressure with air.

Following, the surfaces were analyzed for carbon, oxygen, nitrogen and gold by X-ray photo-electron spectroscopy, of which the results are shown in the table below. Also surfaces that were rinsed with water for 1 hr and subsequently dried were analyzed by XPS.

Table 4

| element | surface composition (at%0) | |
|---|---|---|
| | before rinsing | after rinsing |
| C | 73.4 | 68.3 |
| O | 4.4 | 5.3 |
| N | 8.3 | 9.0 |
| S | 13.3 | 16.5 |
| Au | 0.7 | 0.9 |

**Example 8**

Gold coated substrates (6) were placed in the plasma reactor (see example 2) between the cold electrode on the gas inlet side of the reactor and the hot electrode. The reactor was evacuated to a pressure of less than 0.005 mbar and an air flow of 5 sccm was established through the reactor. After 2 minutes of argon flow the substrates were treated with a dynamic argon plasma (5 sccm, 85 W) for 1 minute and subsequently exposed to an argon flow of 5 sccm for 10 minutes again. Then the argon flow was stopped and after evacuation of the reactor, a mixed flow of allylamine and diallylsulfide (66 v% allylamine) at a pressure of 0.065 mbar was established through the reactor. After two minutes allylamine/diallylsulfide flow the substrates were exposed to ten pulses of 1 second of an allylamine/diallylsulfide plasma at a discharge power of 85 W, the pulses being separated by ten seconds allylamine/diallylsulfide flow. After the final plasma

pulse the allylamine/diallylsulfide flow was continued for 2 minutes after which the flow was discontinued, the reactor was evacuated and subsequently brought to atmospheric pressure with air.

**Example 9**

Gold coated substrates (6) were placed in the plasma reactor (see example 2) between the cold electrode on the gas inlet side of the reactor and the hot electrode. The reactor was evacuated to a pressure of less than 0.005 mbar and an air flow of 5 sccm was established through the reactor. After 2 minutes of air flow the substrates were treated with a dynamic air plasma (5 sccm, 85 W) for 1 minute and subsequently exposed to an air flow of 5 sccm for 10 minutes again. Then the air flow was stopped and after evacuation of the reactor to a pressure less than 0.005 mbar, a diallylsulfide flow at a pressure of 0.025 mbar was established through the reactor. After two minutes diallylsulfide flow the substrates were exposed to ten pulses of 1 second of an diallylsulfide plasma at a discharge power of 85 W, the pulses being separated from each other by ten seconds diallylsulfide flow. After the final diallylsulfide plasma pulse the diallylsulfide flow was continued for 1 minute after which the flow was discontinued and the reactor was evacuated to a pressure less than 0.001 mbar. Then an allylamine flow at a pressure of 0.090 mbar was established through the reactor. After two minutes allylamine flow the substrates were exposed to ten pulses of 1 second of an allylamine plasma at a discharge power of 85 W, the pulses being separated by ten seconds allylamine flow. After the final allylamine plasma pulse the allylamine flow was continued for 2 minutes whereafter the flow was discontinued and the reactor was evacuated to a pressure less than 0.001 mbar and brought to atmospheric pressure with air.

Following, the surfaces were analyzed for carbon, oxygen, nitrogen sulphur and gold by X-ray photo-

electron spectroscopy, of which the results are shown in the table below. Also surfaces that were rinsed with water for 1 hr and subsequently dried were analyzed by XPS.

Table 5

| element | surface composition (at%0) | |
|---|---|---|
| | before rinsing | after rinsing |
| C | 69.8 | 68.3 |
| O | 6.9 | 10.2 |
| N | 14.8 | 12.9 |
| S | 8.5 | 8.6 |
| Au | 0.0 | 0.0 |

**Example 10**

Gold coated substrates (6) were placed in the plasma reactor (see example 2) between the cold electrode on the gas inlet side of the reactor and the hot electrode. The reactor was evacuated to a pressure of less than 0.005 mbar and an argon flow of 5 sccm was established through the reactor. After 2 minutes of argon flow the substrates were treated with a dynamic argon plasma (5 sccm, 85 W) for 1 minute and subsequently exposed to an argon flow of 5 sccm for 10 minutes again. Then the argon flow was stopped and after evacuation of the reactor to a pressure less than 0.005 mbar, a diallylsulfide flow at a pressure of 0.025 mbar was established through the reactor. After two minutes diallylsulfide flow the substrates were exposed to ten pulses of 1 second of an diallylsulfide plasma at a discharge power of 85 W, the pulses being separated from each other by ten seconds diallylsulfide flow. After the final diallylsulfide plasma pulse the diallylsulfide flow

was continued for 1 minute after which the flow was discontinued and the reactor was evacuated to a pressure less than 0.001 mbar. Then an allylamine flow at a pressure of 0.090 mbar was established through the reactor. After two minutes allylamine flow the substrates were exposed to ten pulses of 1 second of an allylamine plasma at a discharge power of 85 W, the pulses being separated by ten seconds allylamine flow. After the final allylamine plasma pulse the allylamine flow was continued for 2 minutes after which the flow was discontinued and the reactor was evacuated to a pressure less than 0.001 mbar and brought to atmospheric pressure with air.

**Example 11**

**Coupling of CMD onto amine functionalized gold surfaces.**

Carboxymethyl cellulose (100 mg) was dissolved in 10 ml 0.05 M 2-(N-morpholino) ethanesulfonic acid after which 5 mg N-hydroxysuccinimid was added. After complete dissolution of this reagent 20 mg N-(3-dimethylaminopropyl)-N' ethylcarbodiimide was added. After 3 minutes activation, an amine functionalized gold surface was incubated with 1 ml of this carboxymethyl dextran solution for 2,5 hours. Then the surfaces were rinsed with phosphate buffered saline, and water and vacuum dried. The whole immobilization procedure was performed at room temperature.

In this example, carboxymethyldextran is used as a model compound for chemical functional group containing compounds in general including but not limited to dextrans including carboxymethyl dextran, carboxymethyl cellulose, mono- di- oligo- and poly-saccharides, gum xanthan, carboxylate and amine dendrimers, and mono-, homo- and hetero-functional carboxylate polyethylene glycols and polyethylene oxide, polyethylene imine, polyacrylic acid, polyvinyl alcohol, etc.

The amount of these functional group containing compounds that is immobilized can be controlled by the

reaction parameters such as reaction time, the concentration of the functional group containing compound and the ratio of coupling agent to functional group containing compound.

**Example 12**

**Immobilization of albumin on a COOH-functionalizes sensing device.**

A sensor device, that was COOH-functionalized by the plasma deposition method was used for the immobilization of albumin. During the immobilization procedure that was performed at 22.5 °C the surface events were monitored by Surface Plasmon Resonance Spectroscopy of which the results are given in figure 1. After mounting the functionalized sensing device in the SPR apparatus, the sensing surface was incubated with 10 mM HEPES buffer for about 5 minutes. Then the HEPES buffer was exchanged for a EDC (20 mg/ml)-NHS (4 mg/ml) solution in water. After 5 minutes activation the EDC/NHS solution was exchanged for an albumin solution (2 mg/ml in 10 mM HEPES) and an immobilization time of 15 minutes was applied. Then the sensing surface was rinsed with HEPES buffer and the stability of the immobilized albumin in HEPES buffer was monitored for 3 minutes after which the rinsing procedure with HEPES buffer was repeated. To study the stability of the immobilized albumin in 0.1 N HCl the HEPES buffer was replaced by 0.1 HCl and the sensing surface was incubated in this solution for 3 minutes after which 0.1 N HCl was replaced for fresh 0.1 N HCl and the measurement was continued for 3 minutes. Then the surface was rinsed with 0.1 N HEPES buffer again an incubation of the sensing surface was proceeded in this buffer for a final 5 minutes.

The results show that upon activation of the sensing surface with EDC/NHS and subsequent immobilization of albumin and rinsing with HEPES buffer the response increases with about 700 milli-degrees indicating the immobilization of albumin on the COOH-

functionalized sensing surface. Rinsing of the surface with 0.1 N HCl only resulted in a decrease of the signal of about 30 milli-degrees, showing that the albumin immobilization is very stable.

The invention is not limited to the above description; the requested rights are rather determined by the following claims.

## CLAIMS

1. Device for investigating reactions between interactive species, said device comprising:

- one or more plasma deposited layers, which layers comprise one or more first pre-selected functional group species, which functional group species are interactible with a pre-selectable second species.

2. Device according to claim 1 wherein the plasma deposited layer is supported on a substrate.

3. Device according to claims 1 or 2 further comprising a film of a free electron metal, preferably selected from the group consisting essentially of copper, silver, aluminum and gold.

4. Device according to claim 3 wherein the plasma deposited layer is arranged directly on the free electron metal film.

5. Device according to any of the previous claims, wherein the plasma deposited layer, comprises one or more chemical and/or biological functional groups.

6. Device according to claim 5, further comprising one or more wet chemically deposited layer(s), arranged on the plasma deposited layer.

7. Device according to any of the preceding claims wherein the plasma layers comprise one or more amine compounds and/or one or more sulphur compounds, preferably thiols, sulfides and/or disulfides and most preferably being diallyl sulfide.

8. Device according to claim 7, wherein the substrate consists essentially of gold.

9. Process for providing a device according to any of the previous claims, comprising the step of depositing a gas plasma layer onto a pre-selected substrate in order to provide the substrate with a predetermined functionality.

10. Process according to claim 9 wherein the plasma layer is directly deposited onto the substrate and/or onto a metal film arranged on the substrate.

11. Process according to claims 9 or 10 wherein plasma is deposited from a monomer/ oligomer/ polymer in gas form, preferably being a monomer, said monomer being saturated, partially saturated or unsaturated.

12. Process according to any of the claims 9-11 wherein the substrate is subjected to a pre-cleaning step comprising pre-treating the substrate by means of a plasma etching step before the plasma deposition step said pre-cleaning step preferably comprising pre-treatment with air plasma.

13. Process according to any of the claims 9-12 wherein the gas plasma is deposited under the following conditions:

- a discharge power of upto 5000 W, preferably upto 500 W,
- an exposure duration of upto 1000 s, preferably upto 100 s,
- a plasma gas flow of upto 10000 $cm^3$/min, preferably upto 100 $cm^3$/min,
- a pressure of upto 1 bar, preferably from between 0,001-50 mbar,
- a frequency covering DC, AC, RF, and the MW, preferably from between 2-60 Mhz.

14. Process according to claim 13 wherein the discharge power is pulsed to the plasma, the pulse discharges being separated by:

- upto 1000 s preferably upto 100 s.

15. Process according to claims 13 or 14 wherein the substrate is treated in an after-glow.

16. Process according to claims 14-15 wherein following pulse discharge, the substrate is after-treated with a pre-selected gas, which gas optionally comprises the one or more functional groups which have been plasma deposited.

17. Process for providing a device according to any of the preceding claims 9-16, suitable for investigating reactions between interactive bio/chemical species by means of surface plasmon resonance spectroscopy, said process comprising the steps of:

- preselecting a free electron metal substrate, which metal substrate is suitable for allowing investigation by surface plasmon resonance spectroscopy, arranging a preselected first functional group species on the free electron metal substrate by means of plasma deposition, which first functional group species protects the free electron metal substrate from a second functional group species whose interaction with the plasma deposited first functional group species can be investigated, thereby preventing undesirable interactions between the free electron metal substrate and the second functional group species, and which first functional group species provides a desired functionality for the second functional group species, and

- subsequently arranging a second functional group species on the plasma deposited layer of the first functional group species, whereafter interaction between the first and second functional group species layers, can be investigated by means of surface plasmon resonance spectroscopy.

18. Process for providing a device according to any of the preceding claims 9-17, suitable for investigating reactions between interactive bio/chemical species by means of surface plasmon resonance spectroscopy, said process comprising the steps of:

- preselecting a free electron metal substrate, preferably being gold, which metal substrate is suitable for allowing investigation by surface plasmon resonance spectroscopy, arranging a preselected first functional group species on the free electron metal substrate by means of plasma deposition, which functional group species preferably is selected from a sulphur compound, which first functional group species protects the free

electron metal substrate from a second functional group species whose interaction with the plasma deposited first functional group species can be investigated, thereby preventing undesirable interactions between the free electron metal substrate and the second functional group species, and which first functional group species provides a desired functionality for the second functional group species.

19. Process according to claim 17 or 18, wherein before being exposed to the second functional group species, a bio/chemical functional layer is wet chemically arranged on the plasma deposited first functional group species layer, said wet chemically arranged functional layer being preselected for its specificity for the second functional group species and for the prevention of non specific interactions with the said second functional group species.

20. Device according to claims 1 to 8, obtainable according to a process according to any of the claims 9-19.

21. Process for investigating the interaction, for example real time surface interaction, of pre-determined chemical and/or biological species, comprising the steps of analyzing the interaction between the species arranged on a device according to any of the claims 1 to 8 and/or 20.

22. Use of a device according to any of the claims 1-8, and/or 20 for investigating the reaction between chemically interactive species, and especially for use in SPR.

23. Use of a device for investigating reactions between interactive bio/chemical species, by means of surface plasmon resins spectroscopy, said device comprising a preselected free electron metal substrate, and a preselected, plasma deposited layer arranged on the free electron metal substrate, which plasma deposited functional group species is chosen for its attachment ability to the free electron metal substrate, and for its

specificity to further functional group species, whereby the interaction therebetween is investigatable by means of surface plasmon resonance spectroscopy.

24. Use of a device according to claim 23, wherein the pre-selected free electron metal substrate consists essentially of gold, and wherein the plasma deposited layer comprises one or more sulphur compounds.

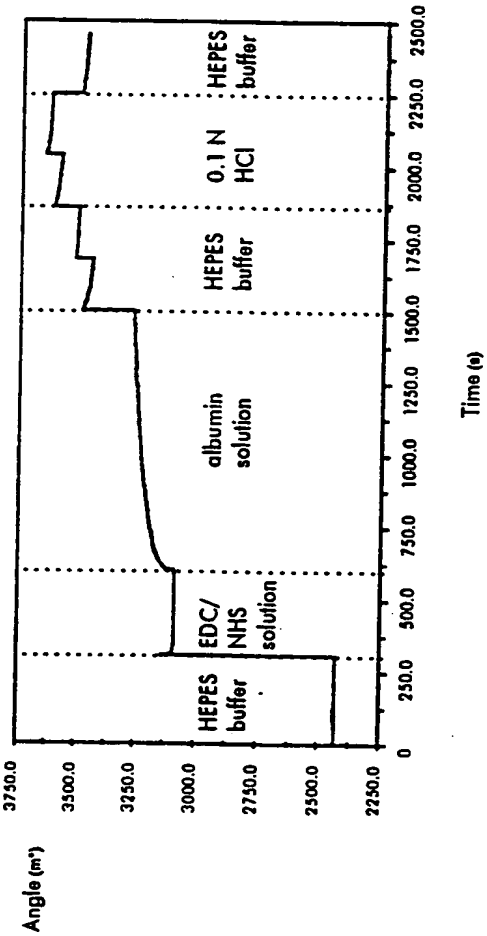
HEPES buffer
0.1 N HCl
HEPES buffer
albumin solution
EDC/ NHS solution
HEPES buffer
Angle (m°)
Time (s)
3750.0
3500.0
3250.0
3000.0
2750.0
2500.0
2250.0
0
250.0
500.0
750.0
1000.0
1250.0
1500.0
1750.0
2000.0
2250.0
2500.0

Figure 1

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**(54) Title:** DEVICE AND PROCESS FOR INVESTIGATING CHEMICAL INTERACTIONS

**(57) Abstract**

The invention relates to a device for investigating reactions between interactive species, said device comprising: one or more plasma deposited layers, which layers comprise one or more first pre–selected functional group species, which functional group species are interactible with a pre–selectable second species.

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|---|---|---|
| E SD/RS/HBG-2 | | |
| International application No. | International filing date *(day/month/year)* | (Earliest) Priority Date *(day/month/year)* |
| PCT/NL 99/ 00504 | 06/08/1999 | 14/08/1998 |
| Applicant | | |
| HOLLAND BIOMATERIALS GROUP B.V. et al. | | |

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

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2. [ ] **Certain claims were found unsearchable** (See Box I).

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[ ] because this figure better characterizes the invention.

[X] None of the figures.

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 99/00504

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/543 B05D7/24

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N B05D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | EP 0 806 250 A (BOEHRINGER MANNHEIM GMBH)<br>12 November 1997 (1997-11-12)<br>the whole document<br>--- | 1-24 |
| X | EP 0 104 608 A (BECTON DICKINSON CO)<br>4 April 1984 (1984-04-04)<br>claims<br>page 3, line 28 -page 4, line 17<br>page 5, line 1 - line 7<br>page 8, line 28 -page 10, line 22<br>--- | 1-24 |
| X | WO 97 38801 A (BOARD OF REGENTS; UNIVERSITY OF TEXAS)<br>23 October 1997 (1997-10-23)<br>claims<br>page 5, line 7 - line 30<br>page 9, line 8 - line 26<br>page 15, line 7 -page 16, line 20<br>---<br>-/-- | 1-24 |

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | US 5 723 219 A (JOHANSON ROBERT G ET AL) 3 March 1998 (1998-03-03) claims 1-6,12-18,24,25,31 | 1,2,5-7, 9-11 |
| Y | column 4, line 65 -column 6, line 67 | 3,4,8, 12-24 |
| | --- | |
| X | US 5 627 079 A (GARDELLA JR JOSEPH A ET AL) 6 May 1997 (1997-05-06) claims column 4, line 49 -column 6, line 24 | 1-24 |
| | --- | |
| X | US 5 266 309 A (GARDELLA JR JOSEPH A ET AL) 30 November 1993 (1993-11-30) | 1,2,5-7, 9-11 |
| Y | claims 12,14 column 3, line 39 -column 4, line 50 column 6, line 17 - line 22 | 3,4,8, 12-24 |
| | --- | |
| X | US 5 055 316 A (FOWLER BRADFORD C ET AL) 8 October 1991 (1991-10-08) | 1,2,5-7, 9-11 |
| Y | claims column 2, line 52 -column 3, line 21 column 4, line 18 -column 5, line 37 | 3,4,8, 12-24 |
| | --- | |
| X | PATENT ABSTRACTS OF JAPAN vol. 098, no. 002, 30 January 1998 (1998-01-30) & JP 09 257797 A (SEKISUI CHEM CO LTD), 3 October 1997 (1997-10-03) | 1,2,5-7, 9 |
| Y | abstract | 3,4,8, 12-24 |
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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
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| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| EP 0806250 A | 12-11-1997 | DE 19618926 A | 13-11-1997 |
| | | JP 10114832 A | 06-05-1998 |
| | | US 5932296 A | 03-08-1999 |
| EP 0104608 A | 04-04-1984 | JP 59080442 A | 09-05-1984 |
| WO 9738801 A | 23-10-1997 | US 5876753 A | 02-03-1999 |
| | | AU 2735597 A | 07-11-1997 |
| | | CA 2253408 A | 23-10-1997 |
| | | CN 1221359 A | 30-06-1999 |
| | | EP 0904157 A | 31-03-1999 |
| US 5723219 A | 03-03-1998 | CA 2213328 A | 26-06-1997 |
| | | EP 0809659 A | 03-12-1997 |
| | | WO 9722631 A | 26-06-1997 |
| | | US 5962138 A | 05-10-1999 |
| US 5627079 A | 06-05-1997 | US 5266309 A | 30-11-1993 |
| | | US 4946903 A | 07-08-1993 |
| US 5266309 A | 30-11-1993 | US 4946903 A | 07-08-1990 |
| | | US 5627079 A | 06-05-1997 |
| US 5055316 A | 08-10-1991 | AU 3533689 A | 24-11-1989 |
| | | EP 0414745 A | 06-03-1991 |
| | | WO 8910377 A | 02-11-1989 |
| JP 09257797 A | 03-10-1997 | NONE | |